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Mutant Prevention Concentrations of Four Carbapenems against Gram-Negative Rods[▽]†

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We tested the propensities of four carbapenems to select for resistant *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* mutants by determining the mutant prevention concentrations (MPCs) for 100 clinical strains with various ß-lactam phenotypes. Among the members of the *Enterobacteriaceae* family and *A. baumannii* strains, the MPC/MIC ratios were mostly 2 to 4. In contrast, for *P. aeruginosa* the MPC/MIC ratios were 4 to ≥16. The MPC/MIC ratios for β-lactamase-positive *K. pneumoniae* and *E. coli* isolates were much higher (range, 4 to >16 μg/ml) than those for ß-lactamase-negative strains.

The mutant prevention concentration (MPC) represents a threshold above which the selective proliferation of resistant mutants is expected to occur only rarely. The ratio of the MPC to the MIC defines the concentration range over which the lower value is the MIC and the upper value is the MPC. Within this range, the growth of susceptible bacteria is suppressed but resistant mutant subpopulations can still be selectively amplified (2, 16, 22, 24, 26, 27). Lower values of the MPC/MIC ratio indicate a better ability to prevent the emergence of mutants (26, 27). MICs and MPCs have been determined for a range of bacterium-drug combinations and, together with knowledge of pharmacokinetic/pharmacodynamic parameters, provide important information on therapeutic outcomes and resistance prevention (17, 19, 21, 28). As far as we know, MPC studies on the activities of carbapenems, such as imipenem, meropenem, ertapenem, and doripenem, against Gram-negative rods have not yet been published. MPCs and MPC/MIC ratios (the mutant selection window hypothesis) are difficult to test clinically (7–9, 25–27). The only published study tested the treatment of *Staphylococcus aureus* with rifampin and confirmed the outcome predicted by the mutant selection window hypothesis. The mutant selection window may be used to design dosing strategies for monotherapy and in the initial application of new compounds. The MPC and MPC/MIC make no contribution if the bacterial population is already fully resistant (7, 8).

The numbers of ß-lactamase-producing members of the *Enterobacteriaceae* family resistant to various β-lactams, extended-spectrum cephalosporins, and even carbapenems are on the rise, as are the numbers of fluoroquinolone- and aminoglycoside-resistant strains (6). There is also an alarming increase in the incidence of multidrug-resistant Gram-negative nonfermenters, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, and strains resistant to all known antibiotics except, in some cases, polymyxin B have appeared (11). In view of such increasing rates of resistance, MPC and MPC/MIC data may be expected to provide information other than that from MIC determinations or the values of pharmacokinetic/pharmacodynamic parameters on the antibacterial activities of carbapenems.

In the current study, the MIC and MPC values of imipenem, meropenem, ertapenem, and doripenem were determined for 100 recent clinical enteric isolates (25 isolates each of *Escherichia coli* and *Klebsiella pneumoniae*) and nonfermenter isolates (25 isolates each of *P. aeruginosa* and *A. baumannii*) with various ß-lactam susceptibilities (MICs) and resistance phenotypes.

The susceptibilities (MICs) of meropenem, imipenem, ertapenem, and doripenem against all clinical isolates of *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* (with the exception of the activity of ertapenem against nonfermenters) were tested by the CLSI agar dilution method (3, 4). The necessary quality control strains were included in each run (3, 4). The MICs and MPCs were interpreted as susceptible or nonsusceptible (intermediate and resistant) for meropenem, imipenem, and ertapenem, according to the CLSI criteria presented in document M100-S19. FDA interpretative criteria (doripenem prescription information) were applied to the MIC results for doripenem (susceptible, ≤0.5 μg/ml for *Enterobacteriaceae*, ≤1 μg/ml for *A. baumannii*, and ≤2 μg/ml for *P. aeruginosa*).

MPCs were determined as follows: pure starter cultures were spread on blood Trypticase soy agar plates (BD Diagnostics, Sparks, MD) (approximately three plates per test isolate), and the plates were incubated overnight at 35°C in air. The cells were then recovered from the plates and resuspended in 0.9% sterile saline. The total numbers of CFU/ml were quantitated on drug-free Mueller-Hinton agar (BD Diagnostics).

For each experiment, agar dilution plates were prepared by incorporating each carbapenem in 2-fold multiples of the initial MIC ranging from 0.25 to 16× MIC into Mueller-Hinton agar plates (BD Diagnostics), and the plates were stored at 4°C for a maximum of 7 days. Imipenem was made fresh daily for each run. Aliquots of 50 μl containing $\geq 10^{10}$ CFU were applied to plain Mueller-Hinton agar plates containing antibiotic. Each drug dilution was tested in duplicate. The inoculated plates were incubated at 35°C in air for 24 h and examined for growth.

Plates with growth in the presence of concentrations at or

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above the determined MIC were analyzed as follows. If >300 colonies (confluent growth) were observed, colonies from four different areas of the analyzed plate were subcultured onto a drug-free sheep blood plate (BD Diagnostics). The bacteria were then suspended in 0.9 ml cation-adjusted Mueller-Hinton broth (BD Diagnostics) and retested by agar dilution in the presence of concentrations at and/or above those concentrations that were initially tested to determine the absence or the presence of resistant mutants. The latter organisms for which the susceptibility values were greater than or equal to the antimicrobial concentration used in the initial selection were labeled mutants. Performance of the agar dilution method confirms the presence or the absence of mutants and allows the elimination of bacterial overcrowding, which may result in an increase in the drug concentration required to prevent the isolation of mutants (2, 5, 15). If ≤300 colonies were observed and the colonies exhibited different morphologies, each colony type was subcultured on a drug-free plate (BD Diagnostics) and tested as described above. In some cases, we observed hazy growth or a thin film, which made accurate reading of the end points difficult. To confirm minimal or no bacterial growth, a loopful of film was scraped from the plate and streaked onto a fresh blood agar plate. If growth occurred, the colonies were further tested by agar dilution to confirm the presence or the absence of mutants on plates containing concentrations at or above the concentration used to select the colonies and retested, and thus, the final MPC was obtained (2, 5). After the cultures were retested for the presence of mutants and the final MPC (the lowest carbapenem concentration that prevented the growth of a resistant mutant subpopulation) was determined, the ratio of the MPC to the MIC was calculated.

The standardized cefazolin (30-μg) Kirby-Bauer disk diffusion method was used for *E. coli* and *K. pneumoniae* isolates to detect β-lactamase activity (3, 4). All β-lactamase-positive enteric isolates were examined for extended-spectrum β-lactamase (ESBL) production by the disk diffusion method with cefotaxime and ceftazidime disks (30 μg each) alone and in combination with a clavulanic acid disk (10 μg), as described previously (18). A decrease in the zone of inhibition (indicated by a ≥5-mm increase in the zone diameter) of either of the antimicrobial agents with clavulanic acid was considered evidence of ESBL production. If the isolates were resistant to either cefotaxime or ceftazidime and were not affected by clavulanic acid, the result of the ESBL test was called indeterminate and the production of AmpC ß-lactamase was suspected phenotypically but was not confirmed genotypically.

The MICs and MPCs for all 100 strains are listed in Table S1 in the supplemental material. The ranges of MICs for the antimicrobials tested were 0.125 to 32 μg/ml for imipenem, 0.016 to 16 μg/ml for meropenem, 0.008 to 16 μg/ml for ertapenem, and 0.03 to 8 μg/ml for doripenem. Among nonfermenter isolates, 8 strains (2 *A. baumannii* and 6 *P. aeruginosa* strains) were non-imipenem susceptible (MICs ≥ 8 μg/ml) and 8 strains (6 *A. baumannii* and 2 *P. aeruginosa* strains) were intermediate to meropenem (MIC = 8 μg/ml), 16 *A. baumannii* strains were non-doripenem susceptible (MICs ≥ 4 μg/ml), and 2 *P. aeruginosa* strains were doripenem nonsusceptible (MICs ≥ 2 μg/ml) (no approved doripenem CLSI breakpoints are currently available; the FDA interpretative criteria were applied). Among the enteric bacteria tested, 15 strains (2 *E. coli* and 13 *K. pneumoniae* strains) were nonertapenem susceptible (MICs ≥ 4 μg/ml), 5 *K. pneumoniae* strains were intermediate to imipenem (MICs = 8 μg/ml), 2 *K. pneumoniae* isolates were non-meropenem susceptible (MICs ≥ 8 μg/ml), and 1 *E. coli* isolate and 13 *K. pneumoniae* isolates were non-doripenem susceptible (MICs ≥ 1 μg/ml).

Previous MIC and MPC studies of the activities of drugs against Gram-positive and Gram-negative bacteria have mainly focused on quinolones, and the MPCs for carbapenems in *Enterobacteriaceae* and nonfermenters have not yet, to our knowledge, been investigated (5, 15–17). Fifteen of the 50 enteric strains (2 *E. coli* and 13 *K. pneumoniae* strains) had elevated doripenem MPCs of 8 to >64 μg/ml (nonsusceptible level, according to the FDA interpretative criteria), with the imipenem and meropenem MPCs being at nonsusceptible breakpoints of 16 to 64 μg/ml and 8 to >64 μg/ml, respectively. Sixteen of the 50 enteric strains (3 *E. coli* and 13 *K. pneumoniae* strains) had ertapenem MPCs at the nonsusceptible level of 8 to >64 μg/ml. Among the *E. coli* strains, 10 were β-lactamase negative and 15 were β-lactamase positive (9 were ESBL producers; 4 were ESBL negative; and the results of the tests for ESBL production were indeterminate for 2, requiring further molecular characterization to specify the type of β-lactamase produced) (see Table S1 in the supplemental material). The presence of both a non-extended-spectrum β-lactamase (as determined by the Kirby-Bauer screening method) and a β-lactamase not affected by clavulanic acid (indeterminate) in two *E. coli* strains resulted in increases of the MPC and the MPC/MIC ratio to ≥4 for all carbapenems tested. This phenotype has not, to our knowledge, been described before. Ten β-lactamase-negative and 15 β-lactamase-positive *K. pneumoniae* strains (5 ESBL producers, 4 ESBL-negative strains, and 6 ESBL-indeterminate strains) were detected (see Table S1 in the supplemental material). The presence of β-lactamase was the common resistance determinant among 13 K. *pneumoniae* strains with nonsusceptible carbapenems MICs and elevated MPCs (4 to >64 μg/ml).

Of the 50 nonfermenters, 41 (20 *A. baumannii* and 21 *P. aeruginosa* strains) had doripenem MPCs greater than or equal to the nonsusceptible breakpoint (≥2 μg/ml for *A. baumannii*, ≥4 μg/ml for *P. aeruginosa*), 36 (12 *A. baumannii* and 24 *P. aeruginosa* strains) had imipenem MPCs greater than or equal to the MPCs for the nonsusceptible range (≥8 μg/ml), and 37 (14 *A. baumannii* and 23 *P. aeruginosa* strains) had meropenem MPCs greater than or equal to the nonsusceptible level (≥8 μg/ml).

The MIC ranges, MIC_{50}s, and MIC_{90}s (μg/ml) for each species can be seen in Table 1. The $(MPC/MIC)_{50}$ ratio was calculated on the basis of the MPC/MIC values reached by 50% of the strains analyzed. The $(MPC/MIC)_{50}$s for the carbapenems were mostly 2 to 4 for *E. coli*, *K. pneumoniae*, and *A. baumannii* and 8 to 16 for *P. aeruginosa.* The $(MPC/MIC)_{90}$ values for the carbapenems were in the range of 8 to 16 for all strains except *E. coli*, for which the $(MPC/MIC)_{90}$ range was 4 to 8.

The number of strains with specific MPC/MIC ratios can be seen in Table 2. The carbapenem MPC/MIC ratios were mostly 2 to 4 against *A. baumannii* isolates, similar to those previously reported for fluoroquinolones (15–17) and for tigecycline and vancomycin (1). Most *P. aeruginosa* strains had carbapenems

TABLE 1. MIC ranges, MIC_{50}s, MIC_{90}s, MPC_{50}s, MPC_{90}s, $(MPC/MIC)_{50}$s, and $(MPC/MIC)_{90}$s for all 100 strains tested

Organism (no. of strains)	Drug	MIC (μg/ml)			MPC (μg/ml)		$(MPC/MIC)_{50}$	$(MPC/MIC)_{90}$
		Range	50%	90%	50%	90%		
Acinetobacter baumannii (25)	Doripenem	0.25–8.0	2.0	4.0	4.0	64.0	2	16
	Imipenem	0.25–8.0	1.0	4.0	4.0	64.0	4	16
	Meropenem	0.5–8.0	2.0	8.0	8.0	128.0	4	16
Pseudomonas aeruginosa (25)	Doripenem	0.125–8.0	0.5	2.0	4.0	16.0	8	8
	Imipenem	0.5–32.0	2.0	8.0	32.0	64.0	16	8
	Meropenem	0.25–8.0	0.5	2.0	8.0	32.0	16	16
Escherichia coli (25)	Doripenem	0.03–2.0	0.03	0.06	0.125	0.25	4	4
	Ertapenem	0.008–8.0	0.03	0.25	0.125	2.0	4	8
	Imipenem	0.125–4.0	0.25	0.5	0.5	2.0	2	4
	Meropenem	0.016–1.0	0.03	0.06	0.06	0.25	2	4
Klebsiella pneumoniae (25)	Doripenem	0.06–4.0	0.125	2.0	0.25	>64.0	2	>32
	Ertapenem	0.008–16.0	0.5	8.0	1.0	64.0	2	8
	Imipenem	0.125–8.0	2.0	8.0	4.0	32.0	2	4
	Meropenem	0.03–16.0	0.06	4.0	0.5	64.0	8	16
Total strains (100)	Doripenem	0.03–8.0	0.5	4.0	4.0	64.0	8	16
	Ertapenem[a]	0.008–16.0	0.06	8.0	0.5	64.0	8	8
	Imipenem	0.125–32.0	1.0	8.0	4.0	64.0	4	8
	Meropenem	0.016–16.0	0.5	4.0	8.0	32.0	16	8

[a] *A. baumannii* and *P. aeruginosa* strains were not included; a total of 50 strains were tested.

MPC/MIC ratios of 8 to >16, which were similar to those reported by Ruzin et al. (20) for piperacillin in combination with BLI-489 and higher than those previously reported for fluoroquinolones (14–17) and tigecycline and vancomycin (1).

The MIC values of meropenem were lower than those of doripenem, imipenem, and ertapenem among the *E. coli* strains. Similar correlations have been reported previously (10, 12, 23). Among the *K. pneumoniae* strains, the meropenem MICs were similar to those of ertapenem but higher than those of doripenem and imipenem. Among the *P. aeruginosa* strains, the meropenem MICs were similar to those of doripenem and higher than those of imipenem. Among the *A. baumannii* strains, the meropenem MICs were similar to those of doripenem and meropenem. Similar correlations have been reported previously (10, 12, 13, 23). The numbers of *P. aeruginosa* strains with meropenem (23 strains), imipenem (24 strains), and doripenem (24 strains) MPCs above the nonsusceptibility breakpoints were similar. The numbers of *A. bau-*

TABLE 2. Distribution of MPC/MIC ratio among all strains tested

Organism (no. of strains)	Drug	No. of strains with MPC/MIC ratios of:					
		1	2	4	8	16	>16
Acinetobacter baumannii (25)	Doripenem	0	15	6	2	2	0
	Imipenem	0	11	9	1	4	0
	Meropenem	0	8	12	1	4	0
Pseudomonas aeruginosa (25)	Doripenem	0	2	3	7	9	4
	Imipenem	0	1	5	7	9	3
	Meropenem	0	0	4	7	6	8
Escherichia coli (25)	Doripenem	0	14	8	2	1	0
	Ertapenem	0	3	11	2	7	2
	Imipenem	0	7	14	4	0	0
	Meropenem	0	13	9	1	1	1
Klebsiella pneumoniae (25)	Doripenem	1	11	4	1	1	7
	Ertapenem	0	8	12	5	0	0
	Imipenem	2	8	10	2	1	2
	Meropenem	1	8	6	6	2	2
Total strains (100)	Doripenem	1	42	21	12	13	11
	Ertapenem[a]	0	11	23	7	7	2
	Imipenem	2	27	38	14	14	5
	Meropenem	1	29	31	15	13	11

[a] *A. baumannii* and *P. aeruginosa* were not included; a total of 50 strains were tested.

mannii strains with MPCs above the nonsusceptibility breakpoints for imipenem (12 strains) and meropenem (14 strains) were similar and lower than those of the non-doripenem-susceptible strains, for which the MPCs were ≥2 μg/ml (20 strains).

Our study may shed light on what carbapenem dose based on the drug MPC may possibly both reduce overall bacterial numbers during infections and restrict the selective amplification of resistant subpopulations that may be present as a part of the total bacterial burden. More work is necessary to test these hypotheses.

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REFERENCES

1. **Blondeau, J. M., S. D. Borsos, and S. E. Sanche.** 2009. Comparative minimum inhibitory concentration (MIC) or mutant prevention concentration (MPC) values for tigecycline (Tig) and vancomycin (Van) tested against toxin positive *Clostridium difficile* (CD) clinical isolates, poster E-208. Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
2. **Blondeau, J. M., X. Zhao, G. Hansen, and K. Drlica.** 2001. Mutant prevention concentrations of fluoroquinolones for clinical isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **45:**433–438.
3. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M07-A8. Eighth edition. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **Clinical and Laboratory Standards Institute.** 2009. Performance standards for antimicrobial susceptibility testing. Approved standard M100-S19. Nineteenth informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Credito, K., K. Kosowska-Shick, P. McGhee, G. A. Pankuch, and P. C. Appelbaum.** 2010. Comparative study of the mutant prevention concentrations of moxifloxacin, levofloxacin, and gemifloxacin against pneumococci. Antimicrob. Agents Chemother. **54:**673–677.
6. **Drawz, S. M., and R. A. Bonomo.** 2010. Three decades of beta-lactamase inhibitors. Clin. Microbiol. Rev. **23:**160–201.
7. **Drlica, K., and X. Zhao.** 2008. Mutant selection window hypothesis: a framework for anti-mutant dosing of antimicrobial agents, p. 101–106. *In* V. Georgiev (ed.), Frontiers in research 2006. National Institute of Allergy and Infectious Diseases. NIH vol. 1. Humana Press, Totawa, NJ.
8. **Drlica, K., X. Zhao, J.-Y. Wang, M. Malik, T. Lu, S. Park, X. Li, and D. Perlin.** 2008. An anti-mutant approach for antimicrobial use, p. 371–400. *In* I. Fong and K. Drlica (ed.), Antimicrobial resistance and implications for the 21st century. Springer, New York, NY.
9. **Drlica, K., and X. Zhao.** 2007. Mutant selection window hypothesis updated. Clin. Infect. Dis. **44:**681–688.
10. **Eraksoy, H., A. Basustaoglu, V. Korten, H. Kurt, R. Ozturk, S. Ulusoy, A. Yaman, A. Yuce, and P. Zarakolu.** 2007. Susceptibility of bacterial isolates from Turkey—a report from the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) program. J. Chemother. **19:**650–657.
11. **Falagas, M. E., and P. Kopterides.** 2006. Risk factors for the isolation of multi-drug-resistant Acinetobacter baumannii and Pseudomonas aeruginosa: a systematic review of the literature. J. Hosp. Infect. **64:**7–15.
12. **Fraenkel, C. J., M. Ullberg, S. Bernander, E. Ericson, P. Larsson, J. Rydberg, E. Tornqvist, and A. Melhus.** 2006. In vitro activities of three carbapenems against recent bacterial isolates from severely ill patients at Swedish hospitals. Scand. J. Infect. Dis. **38:**853–859.
13. **Fritsche, T. R., M. G. Stilwell, and R. N. Jones.** 2005. Antimicrobial activity of doripenem (S-4661): a global surveillance report (2003). Clin. Microbiol. Infect. **11:**974–984.
14. **Hansen, G. T., K. Metzler, K. Drlica, and J. M. Blondeau.** 2003. Mutant prevention concentration of gemifloxacin for clinical isolates of *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **47:**440–441.
15. **Hansen, G. T., X. Zhao, K. Drlica, and J. M. Blondeau.** 2006. Mutant prevention concentration for ciprofloxacin and levofloxacin with *Pseudomonas aeruginosa*. Int. J. Antimicrob. Agents **27:**120–124.
16. **Hermsen, E. D., L. B. Hovde, G. N. Konstantinides, and J. C. Rotschafer.** 2005. Mutant prevention concentrations of ABT-492, levofloxacin, moxifloxacin, and gatifloxacin against three common respiratory pathogens. Antimicrob. Agents Chemother. **49:**1633–1635.
17. **Hesje, C. K., G. S. Tillotson, and J. M. Blondeau.** 2007. MICs, MPCs and PK/PDs: a match (sometimes) made in hosts. Expert Rev. Respir. Med. **1:**7–16.
18. **Jacoby, G. A., and P. Han.** 1996. Detection of extended-spectrum beta-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. J. Clin. Microbiol. **34:**908–911.
19. **Livermore, D. M.** 2003. Overstretching the mutant prevention concentration. J. Antimicrob. Chemother. **52:**732.
20. **Ruzin, A., P. J. Petersen, and C. H. Jones.** 2010. Resistance development profiling of piperacillin in combination with the novel β-lactamase inhibitor BLI-489. J. Antimicrob. Chemother. **65:**252–257.
21. **Smith, H. J., K. A. Nichol, D. J. Hoban, and G. G. Zhanel.** 2003. Stretching the mutant prevention concentration (MPC) beyond its limits. J. Antimicrob. Chemother. **51:**1323–1325.
22. **Smith, H. J., M. Walters, T. Hisanaga, G. G. Zhanel, and D. J. Hoban.** 2004. Mutant prevention concentrations for single-step fluoroquinolone-resistant mutants of wild-type, efflux-positive, or ParC or GyrA mutation-containing *Streptococcus pneumoniae* isolates. Antimicrob. Agents Chemother. **48:**3954–3958.
23. **Turner, P. J.** 2009. MYSTIC Europe 2007: activity of meropenem and other broad-spectrum agents against nosocomial isolates. Diagn. Microbiol. Infect. Dis. **63:**217–222.
24. **Yamamoto, K., K. Yanagihara, K. Sugahara, Y. Imamura, M. Seki, K. Izumikawa, H. Kakeya, Y. Yamamoto, Y. Hirakata, S. Kamihira, and S. Kohno.** 2009. In vitro activity of garenoxacin against *Streptococcus pneumoniae* mutants with characterized resistance mechanisms. Antimicrob. Agents Chemother. **53:**3572–3575.
25. **Zhao, X.** 2003. Clarification of MPC and the mutant selection window concept. J. Antimicrob. Chemother. **52:**731.
26. **Zhao, X., and K. Drlica.** 2002. Restricting the selection of antibiotic-resistant mutant bacteria: measurement and potential use of the mutant selection window. J. Infect. Dis. **185:**561–565.
27. **Zhao, X., and K. Drlica.** 2001. Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. Clin. Infect. Dis. **33**(Suppl. 3)**:**S147–S156.
28. **Zhao, X., W. Eisner, N. Perl-Rosenthal, B. Kreiswirth, and K. Drlica.** 2003. Mutant prevention concentration of garenoxacin (BMS-284756) for ciprofloxacin-susceptible or -resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**1023–1027.